further contemplate that similar loop regions may be identified in other domains of crystal proteins which may be similarly modified through site-specific or random mutagenesis to generate toxins having improved activity, or alternatively, altered insect specificity. In certain applications, the creation of altered toxins having increased activity against one or more insects is desired. Alternatively, it may be desirable to utilize the methods described herein for creating and identifying altered crystal proteins which are active against a wider spectrum of susceptible insects. The inventors further contemplate that the creation of chimeric crystal proteins comprising one or more loop regions as described herein may be desirable for preparing "super" toxins which have the combined advantages of increased insecticidal activity and concomitant broad specificity.

In light of the present disclosure, the mutagenesis of codons encoding amino acids within or adjacent to the loop regions between the α-helices of domain 1 of these proteins may also result in the generation of a host of related insecticidal proteins having improved activity. As an illustrative example, alignment of Cry1 amino acid sequences spanning the loop region between α-helices 4 and 5 reveals that several Cry1 proteins contain an arginine residue at the position homologous to R148 of Cry1C. Since the Cry1C R148A mutant exhibits improved toxicity towards a number of lepidopteran pests, it is contemplated by the inventors that similar substitutions in these other Cry1 proteins will also yield improved insecticidal proteins. While exemplary mutations have been described for three of the loop regions which resulted in crystal proteins having improved toxicity, the inventors contemplate that mutations may also be made in other loop regions or other portions of the active toxin which will give rise to functional bioinsecticidal crystal proteins. All such mutations are considered to fall within the scope of this disclosure.

In one illustrative embodiment, mutagenized *cry1C** genes are obtained which encode Cry1C* variants that are generally based upon the wild-type Cry1C sequence, but that have one or more changes incorporated into or adjacent to the loop regions in domain1. A particular example is a mutated *cry1C-R148A* gene (SEQ ID NO:1) that encodes a Cry1C* with an amino acid sequence of SEQ ID NO:2 in which Arginine at position 148 has been replaced by Alanine.

In a second illustrative embodiment, mutagenized *cry1C** genes will encode Cry1C* variants that are generally based upon the wild-type Cry1C sequence, but that have certain changes. A particular example is a mutated *cry1C-R180A* gene (SEQ ID NO:5) that encodes a Cry1C* with an amino acid sequence of SEQ ID NO:6 in which Arginine at position 180 has been replaced by Alanine.

In a third illustrative embodiment, mutagenized *cry1C** genes will encode Cry1C* variants that are generally based upon the wild-type Cry1C sequence, but that have certain changes. A particular example is a mutated *cry1C.563* gene (SEQ ID NO:7) that encodes a Cry1C with an amino acid sequence of SEQ ID NO:8 in which mutations in nucleic acid residues 354, 361, 369, and 370, resulted in point mutations A to T, A to C, A to C, and G to A, respectively. These mutations modified the amino acid sequence at positions 118 (Glu to Asp), 121 (Asn to His), and 124 (Ala to Thr). Using the nomenclature convention described above, such a mutation could also properly be described as a Cry1C-E118D-N121H-A124T mutant.

In a fourth illustrative embodiment, mutagenized *cry1C** genes will encode Cry1C* variants that are generally based upon the wild-type Cry1C sequence, but that have certain changes. A particular example is a mutated *cry1C.579* gene (SEQ ID NO:9) that encodes a Cry1C* with an amino acid sequence of SEQ ID NO:10 in which mutations in nucleic acid residues 353, 369, and 371, resulted in point mutations A to T, A to T, and C to G, respectively. These mutations modified the amino acid sequence at positions 118 (Glu to Val) and 124 (Ala to Gly). Using the nomenclature convention described above, such a mutation could also properly be described as a Cry1C-E118V-A124G mutant.

In a fifth illustrative embodiment, mutagenized *cry1C** genes will encode Cry1C* variants that are generally based upon the wild-type Cry1C sequence, but that have certain changes. A particular example is a mutated *cry1C.499* gene (SEQ ID NO:11) that encodes a Cry1C* with an amino acid sequence of SEQ ID NO:12 in which mutations in nucleic acid residues 360 and 361 resulted in point mutations T to C and A to C, respectively. These mutations modified the amino acid sequence at position 121 (Asn to

His). Using the nomenclature convention described above, such a mutation could also properly be described as a Cry1C-N121H mutant.

In a sixth illustrative embodiment, mutagenized *cry1C** genes will encode Cry1C* variants that are generally based upon the wild-type Cry1C sequence, but that have certain changes. A particular example is a mutated *cry1C-R148D* gene (SEQ ID NO:3) that encodes a Cry1C* with an amino acid sequence of SEQ ID NO:4 in which Arg at position 148 has been replaced by Asp.

The mutated genes of the present invention are also definable by genes in which at least one or more of the codon positions contained within or adjacent to one or more loop regions between 2 or more α-helices contain one or more substituted codons. That is, they contain one or more codons that are not present in the wild-type gene at the particular site(s) of mutagenesis and that encode one or more amino acid substitutions.

In other embodiments, the mutated genes will have at least about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, or even about 50% or more of the codon positions within a loop region between 2 α-helices substituted by one or more codons not present in the wild-type gene sequence at the particular site of mutagenesis and/or amino acid substitution. Mutated *cry1C** genes wherein at least about 50%, 60%, 70%, 80%, 90% or above of the codon positions contained within a loop region between 2 α-helices have been altered are also contemplated to be useful in the practice of the present invention..

Also contemplated to fall within the scope of the invention are combinatorial mutants which contain two or more modified loop regions, or alternatively, contain two or more mutations within a single loop region, or alternatively, two or more modified loop regions with each domain containing two or more modifications. *cry1C** genes wherein modifications have been made in a combination of two or more helices, *e.g.*, α-helices 1 and 2a, α-helices 2b and 3, α-helices 3 and 4, α-helices 4 and 5, α-helices 5 and 6, α-helices 6 and 7, and/or modifications between α-helix 7 and β-strand 1, are also important aspects of the present invention.

As an illustrative example, a mutated crystal protein that the inventors designate Cry1C-R148A.563. contains an arginine to alanine substitution at position 148, as well as